National Institute of Diabetes and Digestive and Kidney Diseases
Workshop on Noninvasive Measurement of Iron

Session 1: Clinical Needs for Measurement of Iron

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Outline:

I. Quantitative distribution of body iron in health and disease
II. Methods for the assessment of body iron
    A. Direct
    B. Indirect
III. Clinical need for measurements of tissue iron
    A. Established
        1. Management of chelation therapy for iron overload: Cooley's anemia (thalassemia major), sickle cell disease, aplastic and other refractory anemia, myelodysplasia
        2. Diagnosis of iron overload: Primary iron overload disorders
    B. Investigational
        1. Total body iron burden and clinical manifestations in iron overload disorders
        2. Organ iron deposition and toxicity in iron overload: heart, liver, pancreas and other endocrine organs, pituitary, brain iron deposition
        3. Abnormalities of brain iron in neurological disorders

Summary: Physicians have an urgent clinical need for a quantitative means of measuring body storage iron that is accurate, safe, non-invasive and readily available to improve the diagnosis and management of patients with iron overload, including hereditary hemochromatosis, Cooley's anemia (thalassemia major), sickle cell disease, aplastic anemia, myelodysplasia and other disorders. In most circumstances, ***iron deficiency*** can be diagnosed using tests on samples of peripheral blood (serum transferrin receptor concentration/log serum ferritin concentration). In contrast, reliable means of detecting the presence and measuring the extent of ***iron overload*** are lacking. The reference method for evaluation of body iron stores in iron overload is chemical measurement of the hepatic iron concentration in a biopsy sample. The discomfort and risk of biopsy preclude the use of biopsy for frequent serial monitoring of the progress of iron-chelating therapy in chronically transfused patients and limit the use of biopsy in the diagnosis of hereditary hemochromatosis. In addition to immediate clinical application, improved means for non-invasive measurement of iron overload would provide important new information about (i) the relationship about total body iron burden and clinical manifestations in iron overload disorders, (ii) organ iron deposition and iron toxicity, and (iii) abnormalities of brain iron in neurological disorders, includingAlzheimer's disease, prion diseases, and Parkinson's disease.

I. Quantitative distribution of body iron in health and disease

Iron is an essential nutrient required by every human cell. Under physiologic conditions, the concentration of iron in the human body is carefully regulated and normally maintained at about 40 mg Fe/kg body weight in women and about 50 mg Fe/kg in men, distributed between functional, transport and storage compartments, as shown in Table 1 (Brittenham, 2000). Iron balance is the result of the difference between the amounts of iron that are taken up by and lost from the body. Because humans are unable to excrete excess iron, iron balance is physiologically regulated by the control of dietary iron absorption.

Iron deficiency designates conditions in which the body iron is decreased and arises from a sustained increase in iron requirements (due to blood loss, pregnancy or growth) over iron supply. With iron deficiency, the body is unable to produce sufficient amounts of heme, other iron-porphyrin complexes, metalloenzymes, or other iron-containing compounds to sustain normal function.

Iron overload arises from a sustained increase in iron supply over iron requirements and develops with conditions in which the regulation of intestinal iron absorption is altered (hereditary hemochromatosis, refractory anemia with ineffective erythropoiesis) or bypassed (transfusional iron overload), or both. Iron overload results primarily in an increase in storage iron, from the normal range of less than one gram in an adult to sixty grams or more (Figure 1); functional iron is little affected. Whether derived from increased absorption of dietary iron or from transfused red blood cells, progressive iron accumulation eventually overwhelms the body's capacity for safe storage. In all varieties of systemic iron overload, the development and severity of organ damage is closely correlated with the magnitude of the body iron excess. Symptomatic patients may present with any of the characteristic manifestations of systemic iron overload: liver disease with the eventual development of cirrhosis and, often, hepatocellular carcinoma, diabetes mellitus, gonadal insufficiency and other endocrine disorders, arthropathy and increased skin pigmentation; iron-induced cardiomyopathy may be lethal.

Table 1.
Distribution of Iron in the Normal Adult

Type of iron	Concentration (mg/kg)
Functional iron	
Hemoglobin	28-31
Myoglobin	5
Heme enzymes	1
Non-heme enzymes	1
Transport iron	
Transferrin	<1 (0.2)
Storage iron	
Ferritin	4-8
Hemosiderin	2-4
Total	40-50

Figure 1.
Range of Body Iron Stores in Iron Overload

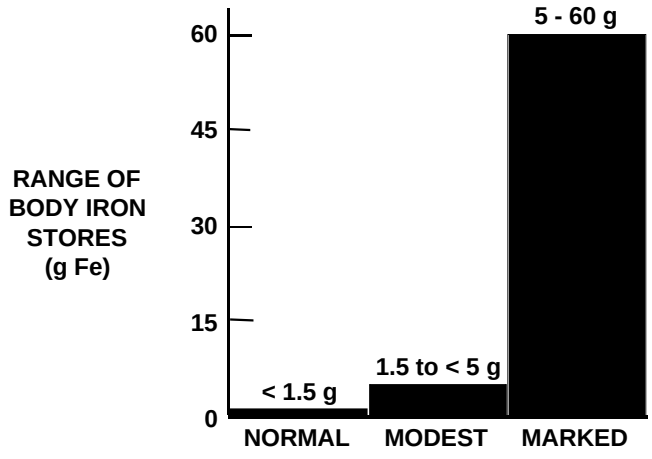
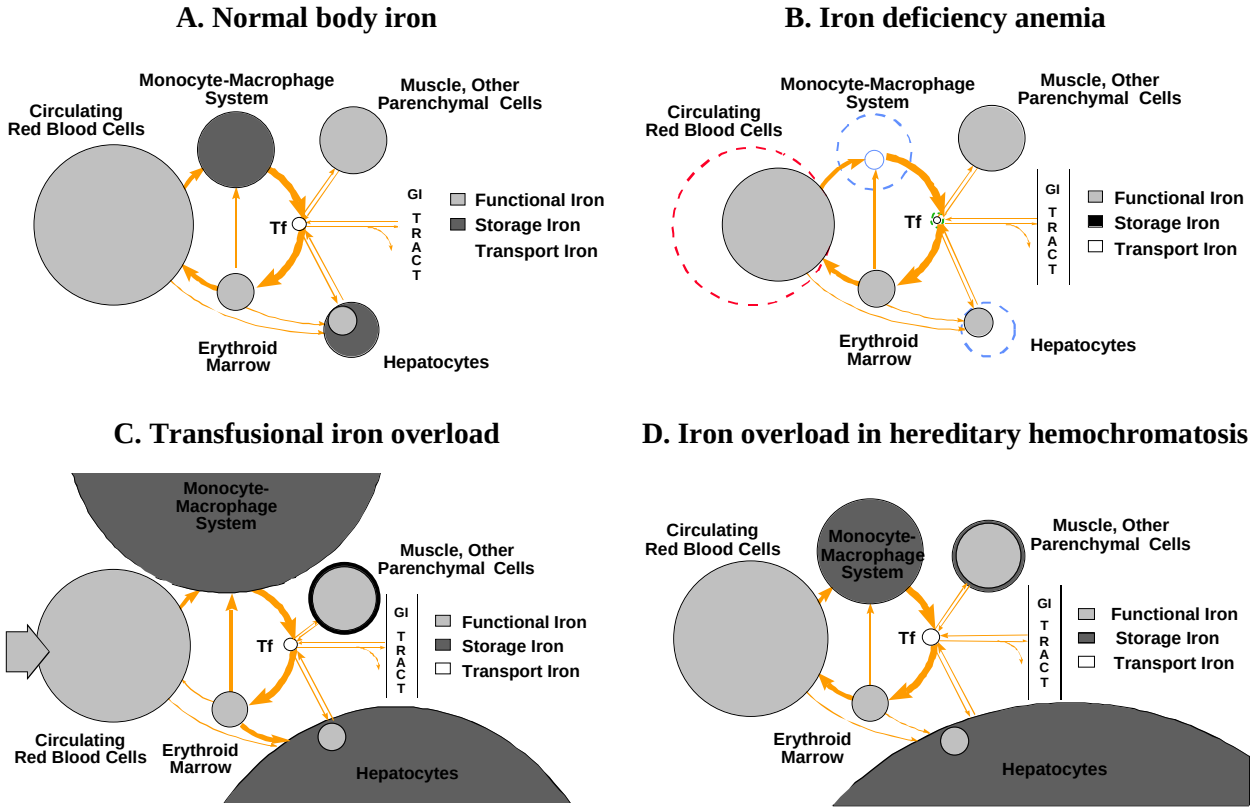


Figure 2 shows schematically the major iron pools (functional, storage and transport) and the major routes of iron movement in a normal adult, in iron deficiency anemia, and in iron overload from chronic transfusion and from hereditary hemochromatosis (Brittenham, 2000). In iron deficiency anemia, both macrophage and hepatocyte iron stores are absent. In transfusional iron overload, marked iron deposition develops in macrophages in the liver (Kupffer cells), bone marrow and spleen, in hepatocytes and, eventually, in other parenchymal cells. In hereditary hemochromatosis, despite massive iron deposition in hepatocytes and, eventually, in other parenchymal cells, macrophage iron stores in the liver, bone marrow and spleen may be only slightly increased, normal or even absent.

Figure 2: Body iron supply and storage (A) in health, (B) in iron deficiency anemia, and in iron overload from (C) transfusion and from (D) hereditary hemochromatosis.



II. Currently available methods for the clinical assessment of body iron: In this section, the discussion will be confined to the assessment of body iron status in patients with iron overload and will consider only established and generally available methods. At present, most cases of iron deficiency seem to be detectable by using the ratio of the serum transferrin concentration to the log of the serum ferritin concentration, determined in a sample of peripheral blood (Punnonen, 1997).

A. Direct: The direct measures of body iron status yield quantitative, specific, and sensitive determinations of body or tissue iron stores in patients with iron overload. *Quantitative phlebotomy* provides a direct measure of total mobilizable storage iron (Angelucci et al., 2000) but is generally acceptable only if the procedure is of therapeutic benefit and cannot be used in transfusion-dependent patients with iron overload. In hereditary hemochromatosis, quantitative

phlebotomy provides a retrospective assessment of the extent of body iron and can be used diagnostically. *Tissue biopsy* of the major iron storage sites, the liver and bone marrow, may provide either qualitative (histologic) or quantitative (chemical analysis) means of ascertaining iron status. The liver is the only storage compartment whose iron content is consistently increased in all forms of systemic iron overload. As shown in Figures 2C and 2D, excess storage iron is detectable in the liver in both parenchymal (in hepatocytes) and reticuloendothelial (in Kupffer cells) cells. **Quantitatively, measurement of the hepatic storage iron concentration provides the best means of evaluating the extent of body iron excess in all forms of iron overload, recognizing that the exact relationship between hepatic iron and the total body iron burden depends on the underlying disorder.** With adequate liver specimens (tissue weight ≥ 1.0 mg, dry weight) and in the absence of cirrhosis or focal lesions, chemical analysis of specimens obtained by liver biopsy is highly reproducible. For example, in a study of duplicate biopsies (N = 16) in patients with thalassemia major, the correlation between duplicates over the range from normal (<480 μg Fe/g, wet weight [<1.6 mg Fe/g liver, dry weight]) to about 9,000μg Fe/g, wet weight [30 mg Fe/g liver, dry weight] was R = 0.99, with a mean difference between duplicates of only 79 ± 140 (S.D.) μg Fe/g, wet weight [0.3 ± 0.5 mg Fe/g liver, dry weight]) (Angelucci et al., personal communication). The usefulness of the hepatic iron concentration in monitoring body iron was shown in a recent study in which patients cured of thalassemia major by bone marrow transplantation underwent quantitative phlebotomy to determine their total body iron stores (Angelucci et al., 2000). With adequate liver specimens (tissue weight ≥ 1.0 mg, dry weight) and in the absence of cirrhosis or focal lesions, the correlation between hepatic iron and total body iron was highly significant ($R = 0.99$, $P < 0.0001$). While biopsy techniques with chemical analysis of tissue iron content provide the most quantitative direct measures of iron status generally available, their discomfort, and for liver biopsy, risk, limit their acceptability to patients and preclude their frequent use in serial observations.

B. Indirect: The indirect measures of body iron status have the advantages of ease and convenience, but clinical experience has shown that these indirect methods may often be misleading in patients with iron overload. All are subject to extraneous influences and lack specificity, sensitivity, or both. The measurement of *plasma ferritin* provides the most useful indirect estimate of body iron stores, but there are many common clinical conditions in which the plasma ferritin is not a reliable indicator of body iron stores. Inflammation, infection, liver disease, hemolysis, ineffective erythropoiesis and ascorbate deficiency – common complications of hereditary hemochromatosis, transfusional iron overload, or both -- all perturb serum ferritin levels independently of changes in total body iron. In particular, normal serum ferritin levels in precirrhotic hemochromatosis restrict their use as a dependable means of detecting increased body iron in this disorder. In patients with severe transfusional iron overload and thalassemia major, the correlation between serum ferritin and iron stores has been characterized as a "fortuitous addition of the effects of iron levels on ferritin synthesis and the effect of cell damage on ferritin release from the liver" (Worwood et al., 1980). The *plasma iron*, *transferrin* and *transferrin saturation* do not quantitatively reflect body iron stores. The lability of plasma iron and transferrin saturation, especially in early hemochromatosis, limit their usefulness as a screening device (Beutler et al., 2000).. Measurement of *urinary iron excretion after injection of an iron chelator* does not quantitatively reflect the level of body iron stores. *Erythrocyte protoporphyrin levels* or *examination of the erythrocyte indices and morphology* are of no use in the detection of iron overload.

III. Clinical need for measurements of tissue iron: In this section, the clinical need for accurate measurement of body iron will be reviewed, considering first, established indications and second, uses of body iron measurement that are still under investigation.

A. Established applications

1. Management of chelation therapy for transfusional iron overload in Cooley's anemia (thalassemia major), sickle cell disease, aplastic and other refractory anemia, myelodysplasia. Transfusional iron overload progressively develops in patients with chronic refractory anemia who require red cell support. In patients with severe congenital anemias, such as thalassemia major (Cooley's anemia), transfusional iron loading begins in infancy. Adequately transfused patients then grow and develop normally during the first decade of life. Thereafter, without treatment for iron excess, growth fails, sexual maturation is delayed or absent, and liver disease, diabetes mellitus, and other endocrine abnormalities develop; patients usually die of heart disease in adolescence. Transfusion-dependent forms of refractory anemia that are acquired later in life, such as aplastic, myelodysplastic, or sideroblastic anemias, ultimately follow a similar course. Heart disease is the most frequent cause of death in patients with all forms of transfusional iron overload. Patients with sickle cell anemia or sickle cell-β thalassemia are also at risk for the complications of iron overload if chronically given transfusions for the prevention of recurrent complications, especially for stroke, as in increasingly the practice (Adams et al., 1998).

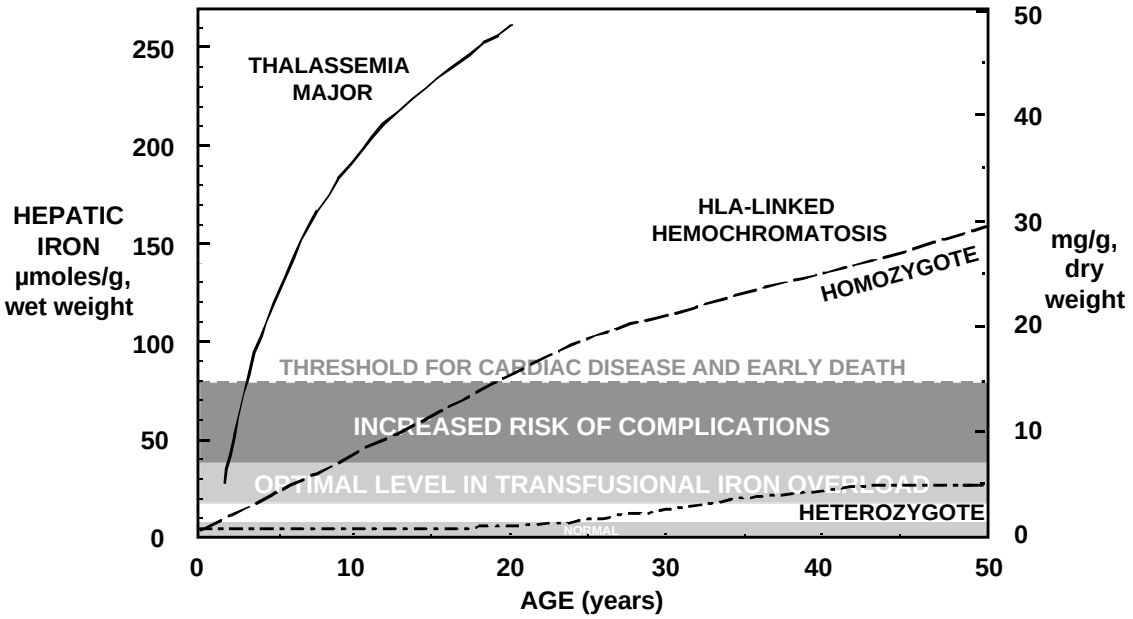


Figure 3: Ranges of hepatic iron concentrations proposed for management of iron chelation therapy in patients with thalassemia major. The solid line labeled "thalassemia major" is derived from studies of patients with thalassemia major treated with red cell transfusion alone, while the interrupted lines labeled "hereditary hemochromatosis" are derived from studies of patients heterozygous or homozygous for hereditary hemochromatosis. The ranges of hepatic iron concentrations shown are (i) those found in normal individuals, labeled "normal"; (ii) those reported in heterozygotes for hereditary hemochromatosis and associated with a normal life expectancy, labeled "optimal in transfusional iron overload"; (iii) those associated with an increased risk of iron-induced complications in patients with transfusional iron overload, labeled "increased risk of complications"; and (iv) the concentration corresponding to a greatly increased risk for cardiac disease and early death in patients with transfusional iron overload, labeled "threshold for cardiac disease and early death". (Adapted from Olivieri and Brittenham, 1997.)

The most important therapeutic goal of iron-chelating therapy in patients with transfusional iron overload is to maintain the body iron in an optimal range that prevents iron toxicity from inadequate chelating therapy while avoiding the side effects of excessive chelator administration (blindness, deafness, growth failure). In patients with transfusional iron overload, the severity of the underlying anemia usually precludes phlebotomy therapy as a means of removing toxic accumulations of iron. Treatment with a chelating agent capable of sequestering iron and permitting its excretion from the body is the only other therapeutic approach now available. Over the past three decades, deferoxamine B (Desferal®) has been found to be a generally safe and effective means of managing iron overload that can prolong survival and avert or ameliorate iron-induced organ damage. Figure 3 shows ranges of hepatic iron concentrations proposed for management of iron chelation therapy in patients with thalassemia major. These ranges may not be directly applicable to patients with other forms of transfusional iron overload (e.g. for aplastic anemia or for sickle cell disease) but are likely to be conservative. For these purposes, the optimal method for iron measurement would have a precision, accuracy and reproducibility similar to that found with duplicate biopsy, to allow reliable discrimination between the ranges of hepatic iron concentrations shown in Figure 3.

2. **Diagnosis of iron overload: Hereditary hemochromatosis and other primary iron overload disorders (juvenile hemochromatosis, transferrin receptor 2-associated iron overload).** The most common genetic disease known among populations of northern European ancestry and the most common form of iron overload in the United States is a genetically determined disorder, the homozygous state for hereditary hemochromatosis, occurring in as much as 0.5 percent of the population or more than 1 million individuals. In hereditary hemochromatosis, the underlying genetic abnormality results in an inappropriately elevated iron absorption, with a chronic progressive increase in body iron stores leading to parenchymal iron accumulation, initially in the liver but later in the pancreas, heart, and other organs. Despite marked parenchymal iron deposition, macrophage iron in the bone marrow may be may be only slightly increased, normal or even absent (Figure 2D). By the time that symptoms of parenchymal damage develop, usually in middle or late life, body iron stores have typically increased from the normal range of 1 g or less to 20 to 40 g or more; further increments in the body iron may be fatal. If hereditary hemochromatosis is diagnosed before irreversible organ damage has developed (cirrhosis, diabetes mellitus), then phlebotomy therapy can remove the excess iron, prevent the development of disease manifestations and give the affected individual a normal life expectancy. The most important recent advance in hereditary hemochromatosis has been the identification in 1996 of the gene, now termed HFE, responsible for the majority of cases (Feder et al., 1996). Mutations in this gene are present in 85% or more of U.S. patients with hereditary hemochromatosis. As a result, hereditary hemochromatosis seems a near ideal candidate for population screening: an autosomal recessive disorder that (i) has a high prevalence in the U.S. population, (ii) has a high frequency of serious clinical manifestations affecting the homozygous genotype, (iii) can be identified by safe and reliable screening and diagnostic tests, and, (iv) after early diagnosis, can be treated effectively and inexpensively to prevent later complications. Despite these favorable factors, not all patients homozygous for HFE mutations have iron overload and not all patients with iron overload have HFE mutations. Thus, while phenotypic and genotypic screening and diagnostic tests can identify those at risk for iron overload, **a major factor limiting the institution of public health programs screening for iron overload is the lack of a reliable, safe, non-invasive and quantitative means of measuring body iron.**

B. Investigational applications: In addition to immediate clinical application, improved means for non-invasive measurement of iron overload would provide important new information in three major areas of investigation.

1. Total body iron burden and clinical manifestations in iron overload disorders. The ranges of hepatic iron concentrations proposed for management of iron chelation therapy in patients with transfusional iron overload, shown in Figure 3, need further validation for patients with thalassemia major and may not be directly applicable to patients with other forms of transfusional iron overload, such as aplastic anemia and sickle cell disease (Nielson et al., 1995; Harmatz et al., 2000; Brittenham et al., 2001).

2. Organ iron deposition and toxicity in iron overload: heart, liver, pancreas and other endocrine organs, pituitary, and joints. The relationships between tissue iron concentration and damage to the heart, liver, pancreas and other endocrine organs, pituitary, and joints, are poorly understood. The development of techniques for the non-invasive measurement of iron concentrations in these organs and tissues would be constitute a major advance in efforts to prevent or reverse these complications of iron overload.

3. Abnormalities of brain iron in neurological disorders. Data are now rapidly accumulating to show that iron and, possibly, other transition metal reactions might be the common denominator underlying Alzheimer's disease, amyotrophic lateral sclerosis, prion diseases, cataracts, mitochondrial disorders and Parkinson's disease (Bush, 2000). The recent demonstration that with a targeted disruption of the gene encoding IRP2 mice misregulate iron metabolism in the intestinal mucosa and the central nervous system (LaVaute et al., 2001) provides powerful support for the hypothesis that abnormalities of brain iron are implicated in the pathogenesis of neurodegeneratve disorders. In adulthood, these mice develop a movement disorder characterized by ataxia, bradykinesia and tremor, with significant accumulations of iron in white matter tracts and nuclei throughout the brain which precede the onset of neurodegeneration and movement disorder symptoms by many months. The development of techniques for the non-invasive measurement of iron concentrations within specific regions of the brain would make a fundamental contribution to efforts to understand the pathophysiologic basis of neurodegenerative disorder.

Conclusion: Physicians have an urgent clinical need for a quantitative means of measuring body storage iron that is accurate, safe, non-invasive and readily available to improve the diagnosis and management of patients with iron overload, including hereditary hemochromatosis, Cooley's anemia (thalassemia major), sickle cell disease, aplastic anemia, myelodysplasia and other disorders. The prognosis in patients with iron overload is influenced by many factors, including the rate and route of iron loading, the age at which iron loading begins; the distribution of iron deposition between macrophage and parenchymal sites; the amount and duration of exposure to circulating non-transferrin-bound iron; ascorbate status; and co-existing disorders, especially alcoholism and viral hepatitis. Nonetheless, the overall magnitude of iron accumulation seems to be the principal determinant of clinical outcome in iron overload. Improved means of measuring body iron would provide important new information about (i) the relationship about total body iron burden and clinical manifestations in iron overload disorders, (ii) organ iron deposition and iron toxicity, and (iii) abnormalities of brain iron in neurological disorders, including Alzheimer's disease, amyotrophic lateral sclerosis, prion diseases, cataracts, mitochondrial disorders and Parkinson's disease.

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